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ADP012184

TITLE: Polymerization in Inverse Microemulsion: An Effective Tool to Produce Biodegradable and non Biodegradable Nanoparticles

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Polymerization in Inverse Microemulsion: An Effective Tool to Produce Biodegradable and non Biodegradable Nanoparticles

Lebon F.^{1,2}, Grandfils C.³, Jérôme R.^{3,4}, Barakat I.³, Sartore L.⁵

¹Dip. Scienze Biomediche e Biotecnologie, Univ. di Brescia, Brescia, Italy

²Ist. Nazionale di Fisica della Materia (INFM), Brescia, Italy

³ Interfaculty Biomaterial Centre, University of Liège, Belgium

⁴CERM (Center for Education & Research on Macromolecules), University of Liège, Belgium

⁵ Dip. di Chimica e Fisica per l'Ingegneria e i Materiali, Università di Brescia, Brescia, Italy

ABSTRACT

Potential of polymerization in inverse microemulsions has been illustrated by the preparation of crosslinked nanoparticles with functional groups on the surface. Nonbiodegradable polyacrylamide nanoparticles have been prepared, with the purpose to use these stable monodisperse lattices as enzymatic reactors and in diagnostic applications. Their size is in the 50 to 90 nm range and they contain a model enzyme (alkaline phosphatase) immobilized. In another example, monodisperse biodegradable nanoparticles of polyamidoamines with a size from 90 to 130 nm have been prepared. They are envisioned for intravenous administration because of a low content of non-metabolized material and absence of toxicity.

INTRODUCTION

The term "microemulsion" has been defined by Danielsson and Lindmann [1] as "a system of water, oil and amphiphile, which is a single phase, optically isotropic and thermodynamically-stable liquid solution". For other workers, a microemulsion refers to dispersion of very small droplets in a medium. In contrast to emulsions, which are opaque, unstable, and contain 1-10 μm droplets, the colloidal particles spontaneously formed in microemulsions are typically up to nanometric-scale globular droplets of the minor solvent, each one surrounded by a surfactant monolayer and thus dispersed in the bulk solvent. The nanoscale compartmentalization in inverse microemulsions (water-in-oil microemulsion, w/o) can be used as a structured reaction medium for the controlled formation of colloids.

Previous papers [2] have reported on an original way to immobilize enzyme within latex particles of reticulated polyacrylamide starting from an inverse microemulsion with a mixture of surfactants AOT (Sodium bis-(ethylhexyl)sulfosuccinate) and Brij30 (polyethylene glycol lauryl ether) in hexane. It is now well-established that the constituents of the reverse micellar system

can modify the interfacial rigidity of the droplets, the interdroplet interaction and ultimately the size and size distribution of the final nanoparticles[3]. The purpose of this work is to show that polymerization in inverse microemulsion can be successfully used for synthesis of calibrated biodegradable and non biodegradable latex particles. We have prepared non-biodegradable nanoparticles of crosslinked polyacrylamide/acrylic acid copolymers, and studied their size and stability after purification and redispersion increasing the functional groups content. The preparation of functionalized biodegradable nanoparticles of biocompatible vinyl-terminated macromonomers based on polyamidoamines was also accomplished and the effect of polymer nature on the particle size was studied.

EXPERIMENTAL DETAILS

Non biodegradable nanoparticles were prepared from a mixture of 18.4 wt % surfactants, 73.8 wt % hexane and 7.8 wt % aqueous phase, i.e. a composition in the microemulsion domain of the pseudo-ternary phase diagram. The organic phase consisted of hexane and a pair of anionic-nonionic surfactants, i.e., AOT and Brij30 in a 34/66 weight ratio. The aqueous phase contained acrylamide (2M), acrylic acid (from 0 to 0.6 M), bis-acrylamide (0.05M) and ammonium persulfate (0.05M) and the enzyme (alkaline phosphatase; 10 µg/ml) in a Tris buffer (pH 10, 0.1M). In a typical polymerization, the aqueous phase was dispersed in the oil phase under stirring and an inert atmosphere was maintained during the whole reaction. The polymerization was initiated by addition of tetraethylmethylenediamine (TEMED) and carried out at 25°C for 30 min. The TEMED/(NH₄)₂S₂O₈ molar ratio was 1/1. Cold acetone (poor solvent) was added to the polymerization medium in order to precipitate the polymer. After centrifugation at 15,000 rpm for 5 min, the upper phase was removed and replaced by fresh acetone. This purification was repeated four times, and the nanolatex was redispersed in water. The aqueous dispersion was immediately dialyzed against water (10,000 cut off dialysis membrane) and then lyophilized. The dry powder was stored at low temperature (4°C).

Biodegradable nanoparticles based on polyamidoamines were prepared by radical polymerization of polyamidoamines oligomers (BAC-2MP and MBA-2MP). These dimacromonomers resulted from polycondensation [4] of an excess of 2,2-bis(acrylamido acetic acid) (BAC) and N,N'-methylenc-bis-acrylamide (MBA), respectively, with 2-methylpiperazine (2MP) (figure 1).

Polymerization proceeded under the same conditions as before, except for the aqueous phase composition. Indeed, the dimacromonomer (2% w/w) and the initiator (ammonium persulfate; 0.5 M) in Tris buffer (0.1 M, pH 8) were dispersed in the organic one under stirring followed by the addition of TEMED (0.3 M in the reaction medium). After reaction under nitrogen at 30°C for 24 h, the nanoparticles were purified by repeated precipitation/redispersion cycles. Then the sample was passed through a membrane of regenerated cellulose with a cut off 100,000 and the dispersion was finally lyophilized.

Table 1: Characterization of polyacrylamide nanoparticles

AA (M)	$\eta^*=[AA]/[Am+AA]$	Particle size (nm)	Zeta potential (mV)
0.0	0.00	55	0.03 ± 0.5
0.1	0.05	77	-11.80 ± 1.9
0.2	0.09	70	-18.15 ± 1.6
0.3	0.15	70	-34.10 ± 2.0
0.4	0.17	158	-29.60 ± 0.6
0.6	0.25	132	-30.40 ± 5.2

*molar ratio of acrylic acid in the comonomers feed

The zeta potential has been measured for the same samples as for PCS. Data in table 2 show that the amount of acrylic acid available on the surface of the particles can be modulated by the composition of the comonomers feed. When the acrylic acid content is increased up to 15 mol%, the zeta potential is more negative and the colloidal stability is improved. Beyond this content, the zeta potential levels off, and the nanoparticle dispersions become less stable.

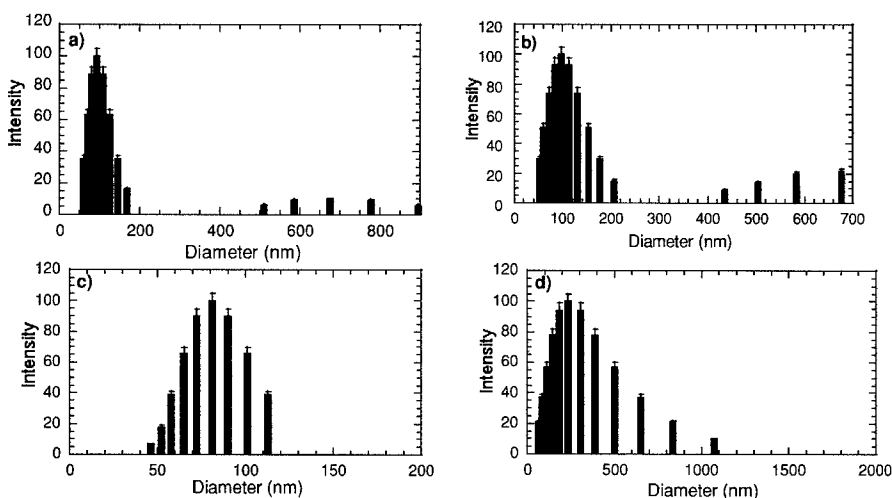
As demonstrated by Candau et al. [6], the acrylic acid has an interfacial behavior and is presumably located at the w/o interface before polymerization. In agreement with Antonietti et al. [7], during the polymerization, a transfert of monomer and comonomer between the interface and the aqueous core could affect the surfactant head group interactions and then the curvature of the interface. At higher concentration in acrylic acid, the transfert is expected to increase involving an increase of the attractive force between particles.

Polymerization in inverse microemulsion has also been carried out in order to prepare biodegradable nanoparticles based on polyamidoamines (PAA). This synthetic polymer consists of regularly alternating amido and tertiary amino groups, and it is water soluble. Radical polymerization of PAA oligomers in inverse microemulsion leads to nanoparticles that can be functional depending on the starting macromonomers. In this work, two series of nanoparticles have been prepared with carboxylic acid group attached to PAA (BAC series) and with non functionalized PAA (MBA series), respectively. M_n and M_w/M_n of the dimacromonomers are listed in table 2.

Table 2: Characterization of dimacromonomers polymerized in inverse microemulsion

	x/y	GPC	
		M_n	M_w/M_n
BAC-2MP	1.41 (I)	725	1,45
	1.16 (II)	1250	1,48
MBA-2MP	1.41 (III)	635	1,51
	1.16 (IV)	1020	1,43

PCS analysis of the BAC-2MP nanoparticles shows a main population with a particle size centered at 107 and 113 nm in case of 30 and 15 mol% of BAC, respectively. A second population of larger particles (> 500 nm) is also observed. Figure 2 shows a population MBA-2MP nanoparticles centered at 91 nm for 30 mol% MBA and very large (340 nm) and polydisperse particles when the MBA content is 15 mol%.

**Figure 2.** Quasi-elastic laser light spectrum of polyamidoamines nanoparticle purified and dispersed in Tris buffer pH=8, 0.1M a) I b) II c) III d) IV

These measurements show that the latex size of the BAC-2MP and MBA-2MP nanoparticles depends on the average molecular weight of the dimacromonomer. As a rule, the

size increases with the molecular weight, and ultimately aggregates can be formed as observed in case of MBA-2MP. Indeed the reaction medium becomes turbid when the sample IV is prepared.

This behavior might be explained by a decrease in the crosslinking density when M_n of the dimacromonomer is increased. This effect was previously reported for crosslinked polyacrylamide nanoparticles [2]. Nevertheless, additional experiments are required to support this explanation. The higher stability of the BAC-2MP nanoparticles suggests that the carboxylic acid groups improve the colloidal stability by electrostatic repulsions.

CONCLUSIONS

Polymerization in inverse microemulsion is an efficient technique to prepare monodisperse nanoparticles of crosslinked polyacrylamide bearing carboxylic acids on the surface. An increase in the acrylic acid content of the comonomers feed up to 15 mol% improves the colloidal stability. Higher content has an adversary effect on the stability, which may lead to latex aggregation. Modulation of either the particle size or the content of carboxylic acid is an important parameter for diagnostic applications. Indeed, occurrence of non selective adsorption of proteins could be prevented and sites for the grafting of sensors can be made available.

Inverse microemulsion polymerization has also proved efficiency for the preparation of biodegradable nanoparticles based on a biocompatible and non toxic polymer: polyamidoamine. Latex prepared from functionalized macromonomer (BAC-2MP) have been prepared with a size of ca. 100 nm.

ACKNOWLEDGMENTS

F. Lebon is indebted to FRIA (Brussels, Belgium) for a PhD grant.

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